Environmental Health Perspectives
Vol. 51, pp. 299-303, 1983

Biochemical Mechanisms in Asbestos Toxicity

by Q. Rahman,* B. Das† and P. N. Viswanathan†

The alarming hazardous nature of asbestos makes it the foremost among toxic fugitive dusts. The biochemical mechanisms responsible for the diverse biological effects of asbestos, such as fibrosis, asbestos bodies, pleural plaques, respiratory difficulty, cancer, and cytotoxicity, are being studied in this laboratory. As asbestosis progresses in guinea pigs, along with reticulum formation, lysosomal enzymes are released from membrane-bound latent state to active free form, initiating degradative changes. Considerable alterations take place in the pulmonary metabolic machinery. Mitochondria in lung cells were found to be important loci for the toxic effect of asbestos. A profile of mitochondrial activity, in control and asbestotic animals, revealed specific enzymic changes such as increased cytochrome c oxidase during the disease. The functional organization of mitochondria was also altered, since the organelles from asbestotic lungs were swollen as measured by spectrophotometry. Glutamate dehydrogenase activity of mitochondria became exposed in asbestosis. The malease dehydrogenase shunt which is involved in transport of the redox potential across the membrane was enhanced in cytosol and mitochondria. The involvement of microsomal enzymes in asbestosis was indicated by alterations in glucose-6-phosphatase and tyrosine transaminase and aniline hydroxylase. Changes in the biotransformational capacity of lung, due to asbestos, could be an important aspect in toxicity, especially the carcinogenic effect. Considerable alterations were encountered in the levels of different phospholipids and in mucopolysaccharide constituents. On the basis of the above, the molecular mechanisms in asbestos toxicity are explained as an integrated model. Interactions of dust constituents with those of membranes and the ensuing metabolic adjustments are thus important in the etiology of asbestosis.

Introduction

Considerable information is available regarding the epidemiological and experimental aspects of the biological effects of asbestos fibers (*1, 2*), but comparatively less is known regarding the chemical and biochemical effects of asbestosis. The available information regarding this aspect has been previously reviewed (*3, 4*). In asbestosis, various biochemical mechanisms are involved in the processes of collagen deposition, damage to plasma membrane of macrophages and erythrocytes, calcification, adsorption of proteins on fibers, enzymatic changes, deficient gas exchange, and alterations in molecular biology. To elucidate these mechanisms in the lung, the target organ, we have conducted a series of studies on the chemical and biochemical aspects of the biological effects of asbestos on the pulmonary system and have summarized their results in the following sections.

Experimental

As asbestosis progresses after intratracheal injection, hydroxyproline and hexosamine contents increase in the lung (Fig. 1), showing changes in collagen and mucopolysaccharides. Simultaneously, histopathological studies revealed marked reticulum fibrosis, with maturation into collagen being slow (*5*).

In the freshly prepared isotonic homogenates from normal animals, acid ribonuclease activity was very low, the full activity being restored only if the homogenate was treated with 0.1% digitonin. However, with the progress of asbestosis, the free activity increased and the activation by detergent decreased. The same pattern was observed with acid DNase, acid phosphatase, and cathepsin. Thus, the normal membrane enclosed latency of lysosomal hydrolases was abolished and the enzyme released into active soluble form in asbestotic lung (Fig. 2) (*6*). This, in turn, could initiate the depletion of func-

*National Center for Toxicological Research, Jefferson, AR 72079.
†Industrial Toxicology Research Centre, Post Box 80, Mahatama Gandhi Marg, Lucknow 226 001, India.

tional and structural components to be eventually replaced by inert fibrous material.

The free activity of acid phosphatase was higher in the cytosol from asbestotic lungs than from control and the presence of dust *in vitro* during homogenation did not break the latency, so that this *in vivo* effect was not a mechanical artifact. Also, the silicic acid could invoke such an effect *in vitro* (unpublished results).

In lung mitochondria (Fig. 3), cytochrome c oxidase activity was higher in asbestotic lung at different stages compared to control (*7*). Asbestos *in vitro* produced inhibition of cytochrome c oxidase, so that a mechanical effect *in vitro* and a chemical effect *in vivo* may be involved. But Ca^{2+}-activated and Mg^{2+}-activated ATPase activities were substantially decreased. Increased cytochrome c oxidase, along with decreased ATPase, could constitute a defensive mechanism to preserve ATP needed for fibrosis.

Figure 4 shows further involvement of mitochondria in asbestosis. The swelling induced by phosphate or calcium and the contraction of ATP and Mg or EDTA was much less in fresh mitochondria from asbestotic lung compared to control (*8*). Thus, mitochondria from asbestotic lung are swollen, which could indicate altered permeability. Malic dehydrogenase, the enzyme involved in shuttling reducing power across mitochondria and cytosol in asbestotic guinea pig lungs, increased as indicated

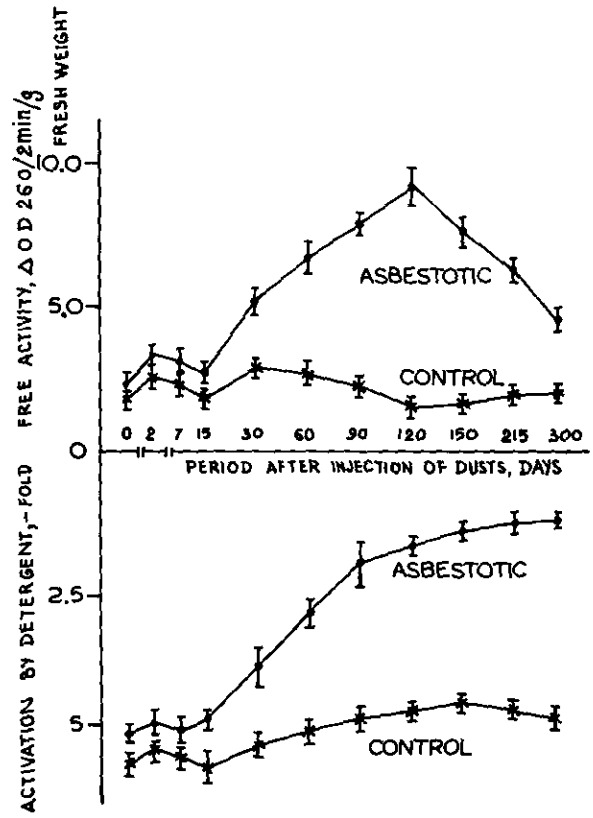

FIGURE 2. Acid ribonuclease activity in control and asbestotic guinea pig lung. From Viswanathan et al. (*6*).

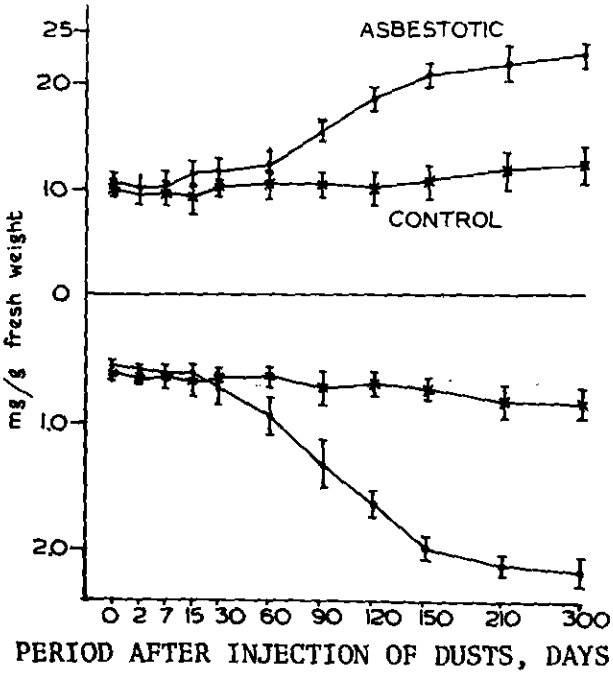

FIGURE 1. Pulmonary fibrogenic response of guinea pigs to 40 mg amosite dust intratracheally. From Viswanathan et al. (*5*).

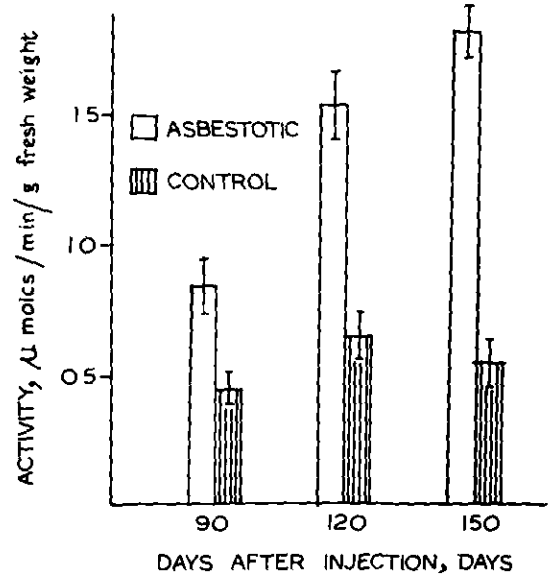

FIGURE 3. Cytochrome c oxidase activity in the mitochondrial fraction of the lungs of normal and asbestotic rats. From Beg et al. (*7*).

(Fig. 5) (*8*). This increase, along with the swollen state, could help in the transport of redox-potential disturbances caused by the dust and increased cytochrome c oxidase to other loci in the cells for various functions. The mitochondrial changes in asbestosis are not confined to terminal oxidase only. The decrease in aconitase and increase in fumarase indicate that the formation of Krebs cycle could be affected (*9*). On the whole, bioenergetic mechanisms in lung undergo marked modulation in response to the metabolic stress by asbestos.

It is evident from Figure 6 that amosite-induced asbestosis resulted in a significant increase in microsomal glucose-6-phosphatase up to 150 days and later decreases. Thus, increased activity of endoplasmic reticulum takes place during the proliferative phase of the disease and is adversely affected during sclerotic phase of fibrosis. Apparently, biotransformation mechanisms are also affected, since glucose-6-phosphatase is a microsomal marker.

Figure 7 shows that at 90, 150 and 210 days after asbestos injection, lactic acid content remained significantly higher than controls (*10*).

Simultaneously, lactic dehydrogenase also increased. Heat denaturation studies showed the increase was due to LDH_5 isozyme. Thus, in asbestosis an increasing trend towards anaerobic metabolism was apparent, which could partly be due to an increased macrophage population. Figure 8 indicates carbonic anhydrase activity of unperfused whole guinea pig lung homogenate was lower than the controls at 40, 80, 120, and 160 days, respec-

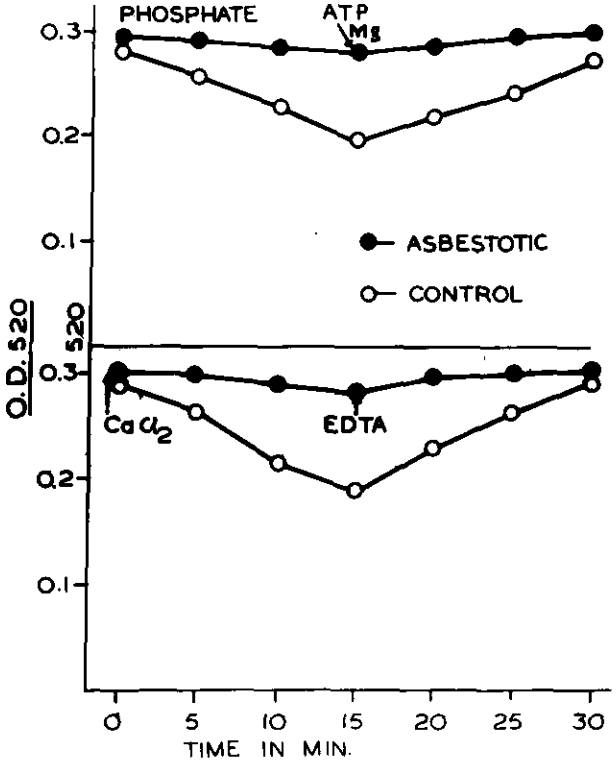

FIGURE 4. Swelling and contraction of mitochondria From normal and asbestotic rat lungs. From Rahman et al. (*8*).

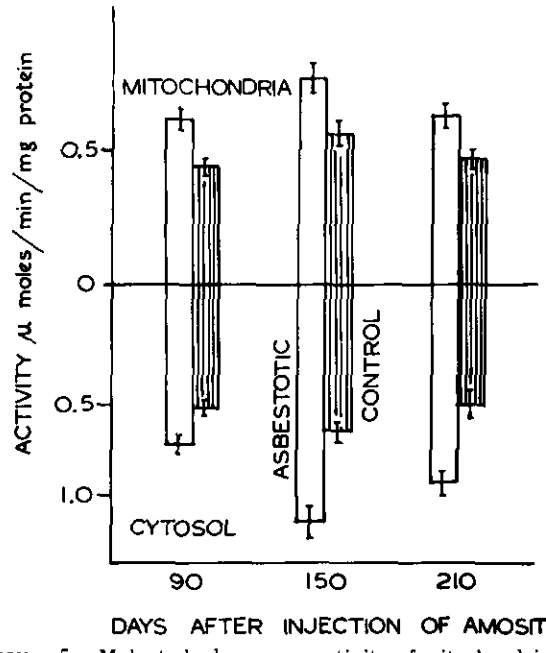

FIGURE 5. Maleate hydrogenase activity of mitochondria and cytosol from normal and asbestotic guinea pig lungs. From Jaiswal et al. (*9*).

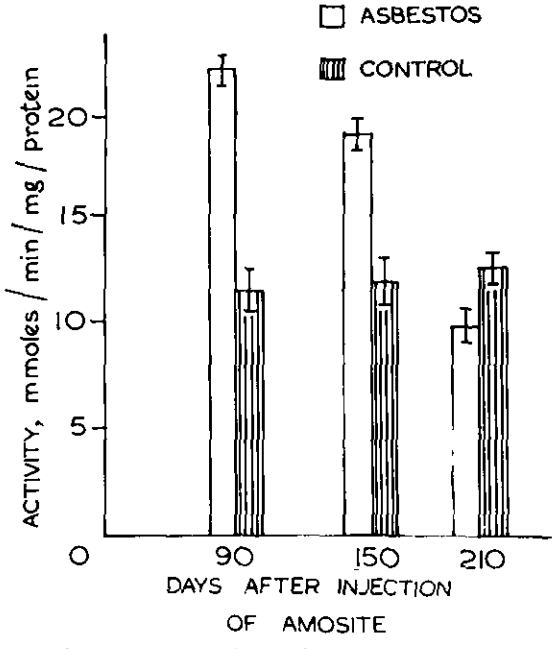

FIGURE 6. Microsomal glucose-6-phosphatase activity of normal and asbestotic guinea pig lungs. From Misra et al. (*10*).

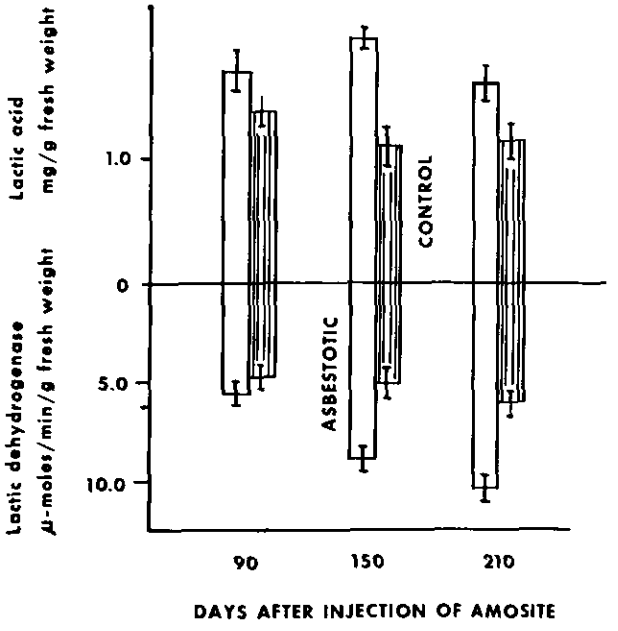

FIGURE 7. Lactic dehydrogenase activity of normal and asbestos-treated guinea pig lungs. From Misra et al. (*10*).

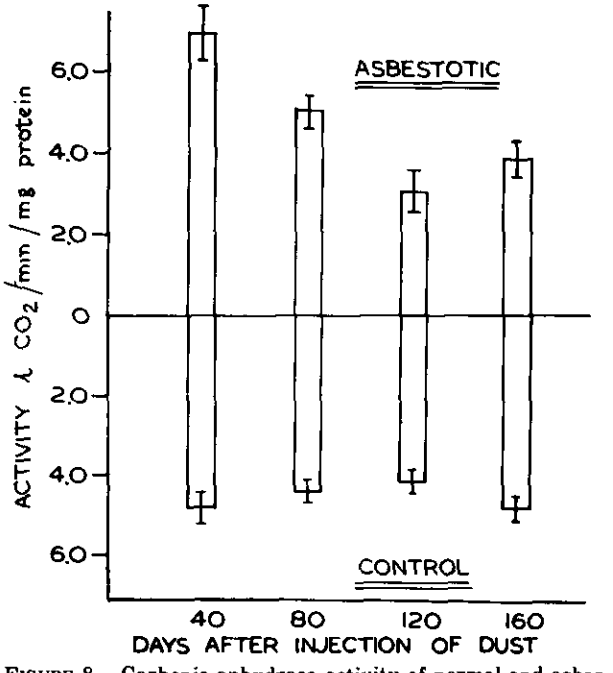

FIGURE 8. Carbonic anhydrase activity of normal and asbestotic guinea pig lung. From Misra et al. (*10*).

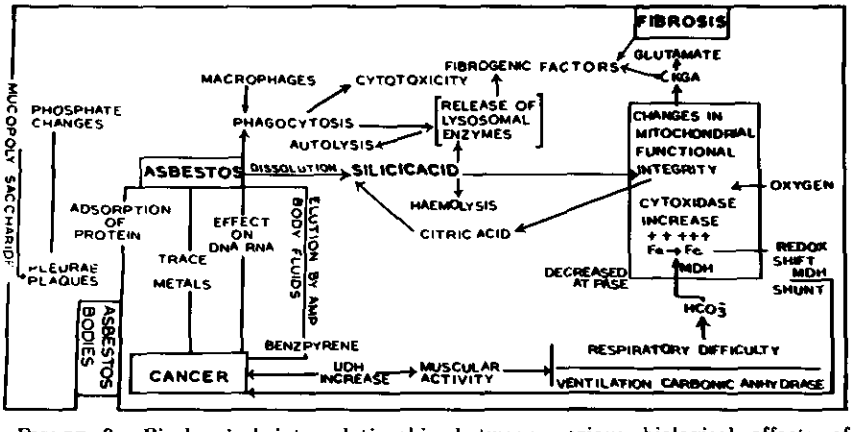

FIGURE 9. Biochemical interrelationship between various biological effects of asbestos.

tively, especially at the earlier stages. This could be involved in the decreased respiratory gas exchange in asbestosis.

Conclusions

Figure 9 summarizes the findings in the form of an integrated biochemical model, explaining the diverse biological effects of asbestos. Fibrosis, pleural plaques, asbestos bodies, respiratory difficulty, and cytotoxicity are related to the damage to biomembranes caused by asbestos. Membrane alterations, especially on mitochondria, will cause shift in redox equilibrium that is reflected outside the mitochondria. This, in turn, stabilizes epoxides (*11*), which are important in carcinogenesis. Also, anaerobic metabolism is increased, as is evident from enhanced LDH, type 5. Further, serum was found to elute trace metals and nucleotides to elute benzo(a)pyrene from asbestos. Moreoever, fibers were found to adsorb DNA and RNA (*12*). These observations also suggest that a number of the early pathogenic changes in asbestosis may have ramifications important for asbestos produced cancer as well as toxicity.

Thanks are due to Dr. Angelo Turturro for his help in preparing the manuscript.

REFERENCES

1. Selikoff, I. J., and Lee, D. H. K. Asbestos and Diseases. Academic Press, New York, 1978.
2. Michaels, L., and Chissick, S. S. Asbestos: Properties, Applications and Hazards (Part 1). Wiley, New York, 1979.
3. Harington, J. S., Allison, J. C., and Badani, D. W. Mineral fibers: chemical, physiological, and biological properties. Advan. Pharmacol. Chemotherap. 12: 291-409 (1975).
4. Rahman, Q. Chemical aspects of the toxicity of silicates. J. Sci. Ind. Res. 36: 312-323 (1977).
5. Viswanathan, P. N., Dogra, R. K. S., Shanker, R., and Zaidi, S. H. Pulmonary fibrogenic response of guinea pigs to amosite dust. Intern. Arch. Arbeits. Med. 31: 51-59 (1973).
6. Viswanathan, P. N., Rahman, Q., Beg, M. U. and Zaidi, S. H. Pulmonary lysosomal enzymes in experimental asbestosis in guinea pigs. Environ. Physiol. Biochem. 3: 120-126 (1973).
7. Beg, M. J., Rahman, Q., Viswanathan, P. N., and Zaidi, S. H. The effect of asbestos dust on mitochondrial enzymes of rat lung. Environ. Physiol. Biochem. 3: 185-191 (1973).
8. Rahman, Q., Viswanathan, P. N., Beg, M. J. and Zaidi, S. H. Involvement of subcellular organelles in the toxic effects of silicate dusts: In: Proceedings of International Symposium on Industrial Toxicology. Lucknow, India (S. H. Saidi, Ed.), INSDOE, New Delhi, 1975, pp. 301-303.
9. Jaiswal, A. K., Sanena, V., Rahman, Q., Viswanathan, P. N., and Zaidi, S. H. Metabolic changes in guinea pig lungs during advanced asbestosis. Indian J. Biochem. Biophys. 15: 37-45 (1977).
10. Misra, V., Rahman, Q., and Viswanathan, P. N., Biochemical changes in guinea pig lungs due to amosite asbestos. Environ. Res. 16: 55-61 (1978).
11. Flower, E. S. Relationship between exposure to asbestos, collagen formation, ferruginous bodies, and carcinomas. Am. Ind. Hyg. Assoc. J. 35: 724-729 (1974).
12. Misra, V., Rahman, Q., Viswanathan, P. N., Beg, M. U., and Zaidi, S. H. 3,4-Benzo(a)pyrene content in chrysotile asbestos and its significance to the biological effects of asbestos. In: Proceedings of International Symposium on Industrial Toxicology, Lucknow, India (S. H. Zaidi, Ed.), INSDOE, New Delhi, 1975, pp. 368-378.